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Influence of sperm-oocyte coincubation period on porcine *in vitro* fertilization (IVF) efficiency

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A major obstacle for successful *in vitro* production of porcine embryos is the polyspermic fertilization. One possibility to reduce polyspermic penetration is decreasing the number of spermatozoa added to the fertilization medium. Unfortunately, the lower rate of polyspermy is accompanied by a reduced penetration rate. A short gamete coincubation period of 10 min has been described to obtain fertilization rates similar to 6 h of coincubation and may improve IVF efficiency (number of monospermic fertilized oocytes/total number inseminated) depending on sperm-oocyte ratio (Gil, 2007, Theriogenology, 67(3), 620–626). Here we demonstrate that the optimal coincubation period in our IVF conditions is between 10 min and 6 h. *In vitro* matured oocytes (n = 600) were inseminated with frozen-thawed epididymal semen with 600 spermatozoa per oocyte and coincubated for 2, 4 and 6 h. At 2 and 4 h post insemination (hpi), oocytes were vortexed and transferred to fertilization medium without spermatozoa. At 6 hpi, presumed zygotes of all groups were washed three times in culture medium and cultured. At 22 hpi, zygotes were fixed overnight and stained with Hoechst 33,342 for the assessment of fertilization and polyspermy. The IVF efficiency was higher for the 4 h group (40 ± 5%) than the 2 and 6 h group (19 ± 8% and 17 ± 5%). Between 4 and 6 h of gamete coincubation, the increase in the number of polyspermic oocytes was relatively higher than the increase in penetration rate (+39% vs. +15%), resulting in a decline in efficiency. (This study was supported by Research Foundation-Flanders).

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Comparison of three semen extenders: tris egg yolk, EQUEX® and LDL (low density lipoproteins) in canine sperm cryopreservation

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Chicken egg yolk is held as an excellent cryoprotective agent for freezing canine semen. Recent advances enabled the extraction of low density lipoproteins from egg yolk, which are responsible for the cryoprotective abilities of the latter. The aim of the study was to compare three semen extenders for freezing canine semen: two containing egg yolk (Tris egg yolk and Equex STAMP) and one containing 6% LDL. After freezing and thawing 20 ejaculates from five different dogs, the 6% LDL extender produced 50% mobile spermatozoa, compared with 48% with the Equex® extender and 27.7% with the extender containing Egg Yolk alone (EY). *In vitro* functional tests demonstrated that plasma membrane integrity (hypoosmotic swelling test) was maintained in 65–66% of spermatozoa as a function of the extender; DNA integrity was maintained in more than 97% of the spermatozoa. The Equex® extender provided superior acrosome integrity (FITC/PSA test): 68.4% compared with 55.1% with LDL and 53.3% with egg yolk. However, the 6% LDL extender resulted in fewer spermatozoal anomalies (Spermac® test), with 54.6% normal spermatozoa compared to 53.6% for Equex® and 53.3% with the egg yolk. All six of the bitches inseminated artificially via the intrauterine route (Scandinavian technique) using semen frozen in the 6% LDL extender became pregnant. The LDL extender resulted in percentages of mobile spermatozoa and movement characteristics that were as good if not better than those obtained with the reference extenders following thawing. The 6% LDL extender appears to have the same cryoprotective qualities as the reference diluent, Equex® STAMP.

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The effect of egg yolk on the survival of sperm from an ai dose after thawing

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Extenders with egg yolk are regularly used for the preservation of bull ejaculate for protection of sperm. Egg yolk influences the stability of the extender to a certain degree. The use of egg yolk as an ingredient of extenders is restricted mainly for hygienic reasons. The sperm activity of an AI dose after thawing by a heat test (38°C, 120 min) was monitored. Two extenders without egg yolk (Andromed, Bioxcell) and two extenders with egg yolk (Triladyl, Optidyl) were used for the prodution of the AI doses. A total of four selected sires used in the system of insemination of dams were evaluated. The bulls were of the same age and had the same quantity and quality characteristics of semen. The results were evaluated by the SAS GLM procedure. Higher activity of sperm after thawing (48.4–51.6%, $p < 0.05$–0.001) was detected in extenders with egg yolk. Sperm activity declined unevenly during the survival test. Sperm activity was significantly higher ($p < 0.01$–0.001) in egg yolk extenders (26.7–31.4%) compared to ones without egg yolk (22.2–25.9%) after 120 min of the survival test. Significant differences in sperm survival among individual sires were determined. Funded by MSMT 6046070901 and QI91A061.

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Urachal calculi in a dalmatian bitch

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Various urachal abnormalities has been documented in several animal species (Laverty P.H., Salisbury S.K.: Surgical management of true patent urachus in a cat. (Journal of Small Animal Practice, 2002, 43, 227–229). One of the developmental abnormalities of the puppy bladder is a persistent urachus. In the fetus, the urachus is a tube-like structure, which, by exiting through the umbilical (navel) area, connects the puppy's bladder to the placental tissues. The most frequent type of urachal disordes are: bladder urachal diverticulum, urachal sinus, urachal ligament, urachal cysts, patent urachus and urachal calculi. A dalmatian bitch 6 years old weighing 10 kg was presented for ovaryohysterectomy. Physical examination of the bitch did not reveal any abnormalities in the navel area. Blood samples were collected from cephalic vein for standard pre-surgery haematology analysis. Surgery was performed to remove the uterus and ovaries by standard method. After premedication with atropine sulphate (Atropina solfato, Ati, Ozzano Emila, Bologna Italy) 0.05 mg/kg, the anaesthesia was induced using a mixture of ketamine (Imalgene100, Merial, Italia) 5 m/Kg and medetomidine (Domitor, Pfizer, Roma, Iyaly) 40 mcg/kg i.m. The anaesthesia was maintained with isoflurane (2%) and oxygen was supplied by a cuffed endotracheal tube. Cephalexin (Mylan, Milano Italy) 15 mg/kg was administered at the time of induction. The bitch was in dorsal recumbency and the abdominal region was prepared for standard surgical procedure. One incision caudally to the umbilicus was performed to remove genital appatatus using a standard technique. The postoperative treatment applied was amoxicillin and clavulanic acid (Synulox Pfizer, Roma, Italy) 10 mg/kg and 2.5 mg/kg respectively every 12 h for 7 days. At the opening of abdominal cavity the apex of the bladder appeared adjoined to the umbilicus by means of a short tubular structure. Inside this structure there was a calculus of 4 mm in diameter. The application of light pressure to the bladder not resulted in urine appearing in the umbilical zone and no umbilical stoma was showed. The bladder was normally developed; the lateral ligament and umbilical arteries were in normal condition. After identification of the ureters, the urachus was isolated with a circumferential ligature